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Studies of Exposure of Rabbits to Electromagnetic Pulsed Fields

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Dutch rabbits were acutely exposed to electromagnetic pulsed (EMP) fields (pulse duration 0.4 μs, field strengths of 1–2 kV/cm and pulse repetition rates in the range of 10 to 38 Hz) for periods of up to two hours. The dependent variables investigated were pentobarbital-induced sleeping time and serum chemistry (including serum triglycerides, creatine phosphokinase (CPK) isoenzymes, and sodium and potassium). Core temperature measured immediately pre-exposure and postexposure revealed no exposure-related alterations. Over the range of field strengths and pulse durations investigated no consistent, statistically significant alterations were found in the end-points investigated.

Key words: electromagnetic pulsed (EMP) fields, pentobarbital-induced sleeping time, serum chemistry, serum triglycerides, creatine phosphokinase (CPK), Dutch rabbits

INTRODUCTION

This study was undertaken to investigate the acute effects of exposure of a mammalian species to electromagnetic pulsed (EMP) fields. This is necessary because humans are exposed to such fields in a number of occupations and there is little data on its effects.

The EMP fields used in this study were short duration (ie, less than a microsecond), high-intensity (ca 2 kV/cm) fields with a characteristic frequency in the megahertz range but consisting of a spectrum of frequencies rather than a discrete frequency. Typically, EMP exposures are intermittent, at irregular repetition rates, and of varying durations. The type of exposures, as well as the EMP characteristics, differ significantly from those generally encountered by humans or experimental animals exposed to microwave or radio-frequency radiation, greatly limiting the applicability of this large body of data in assessing the biological effects of EMP.

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This study was designed to investigate acute effects of EMP exposure on Dutch rabbits, not to assess possible delayed effects. Previous investigations [Wangemann and Cleary, 1976; Cleary and Wangemann, 1976] indicated that certain physiological measures (pentobarbital-induced sleeping time and serum chemistry) were altered by exposure of Dutch rabbits to acute low-intensity (ca 10 mW/cm^2) continuous-wave and pulse-modulated 1.7- and 2.45-GHz fields. This study was designed to permit a comparison of the effects of EMP and microwave exposure in the same species.

METHODS AND PROCEDURES

EMP Exposure

Littermate Dutch rabbits eight to 12 months old were exposed to pulsed capacitive electromagnetic fields in an EMP simulator at the Electromagnetic Radiation Bioeffects Laboratory at the Naval Surface Weapons Laboratory, Dahlgren, Virginia. The EMP simulator consisted of two sheets of copper, 61 X 114 cm. The configuration of the sheets was such that the horizontal section used to expose experimental animals had dimensions of 56 X 61 cm and a plate separation of 19 cm, whereas in the remainder of the plate section, 58 X 61 cm, the gap was narrowed to 6.4 mm and filled with polyethylene to provide the desired capacitance distribution of the simulator. The capacitance of the section separated by the 19-cm air gap was 16 pF and the section separated by the polyethylene sheet was 0.77 nF. The characteristic frequency of the simulator, 23.5 MHz, was determined primarily by the section separated by the polyethylene, such that capacitance changes induced by insertion of experimental animals into the air gap had a minimal effect upon the electrical characteristics of the pulse including the characteristic frequency and the pulse decay time. The EMP pulse, which was triggered by a "free running" spark gap maintained under three atmospheres of nitrogen pressure, had a rise time of less than 0.1 μs followed by an exponentially decaying cosine wave, the amplitude of which decreased to 50% of the initial maxima in approximately four cycles, providing a pulse duration of approximately 0.4 μs. The mean pulse repetition rate (PRR), which was a function of plate voltage, was 24 Hz for a plate voltage of 36 kV (ie, 1.9 kV/cm) or 10 Hz for a voltage of 26 kV (1.37 kV/cm), for example. Since the spark gap was free-running, ie, not triggered, the PRR was not constant during exposures. At a plate voltage of 36 kV the PRR varied from 22 to 25 Hz (mean interpulse duration 42 ms), and at 26 kV the range of PRR was from 8 to 12 Hz (mean interpulse duration 100 ms). The EMP simulator was located within a wire mesh Faraday cage to contain stray radiation.

Dutch rabbits were exposed one at a time for various durations. Four styrofoam panels were placed between the plates to restrict the unanesthetized animals to the central region of the pulsed-field exposure chamber where the electrical field was uniform. The exposure region was of an adequate size to permit the animal to reorient during exposure. Ventilation was provided by holes in the four styrofoam panels and the exposures were conducted at room temperature (22 ± 1 °C). When an anesthetized rabbit was exposed, the subject was placed in the center of the chamber on a plexiglass sheet used to electrically insulate the plates of the EMP simulator. During sham exposures animals were placed in a mock-up EMP exposure chamber located immediately outside the Faraday cage, within one meter of the simulator. Sham exposures occurred simultaneously with EMP exposure. This procedure was necessary to reduce the possibility of artifacts due to ozone and noise produced by the operation of the spark-gap trigger of the pulser.

Pentobarbital-Induced Sleeping Time

The effect of repeated EMP exposure at PRRs of 10 and 24 Hz on drug-induced sleeping time was investigated using a technique employed in studies of the analeptic effect of exposure to 1.7- and 2.45-GHz microwave radiation [Cleary and Wangemann, 1976]. Littermate Dutch rabbits, 10 to 14 months old, were anesthetized with 22 mg/kg of pentobarbital, a short-to-intermediate duration, nonselective central nervous system depressant. Injection of this anesthetic into the auricular artery of the rabbit ear induces rapid (ie, within 30 seconds) anesthesia characterized by loss of the righting reflex, the criteria used to define the start of the sleeping time. The time duration for the rabbit to regain its righting reflex is defined as the sleeping time. Rectal (deep-colonic) temperatures were measured with a thermistor probe and a digital thermometer with a time constant of eight seconds. Temperature was measured immediately prior to anesthesia and immediately after the animal regained the righting reflex.

The results of previous studies indicated a statistically significant correlation between sleeping time and body weight in the Dutch rabbit [Cleary and Wangemann, 1976]. This indicated the need to use animals of similar body weight and age. In order to decrease the effects of variations in size and because only a small number of animals could be exposed to the pulsed field during a given experimental session, it was necessary to conduct two separate experiments over a one-year period. Exposure effects in this and in all other experiments in this study were evaluated by comparison of treatment and sham means using a two-tailed Student's t-test.

Serum Components

A study was undertaken of the effects of acute exposure of Dutch rabbits to EMP fields using as the dependent variables: Calcium, inorganic phosphate, glucose, blood urea nitrogen (BUN), uric acid, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase (LDH), and serum glutamic oxaloacetic transaminase (SGOT).

Ten days prior to EMP or sham exposure, base-line values were determined from 5-ml blood samples obtained by venipuncture of the marginal ear veins of ten littermate Dutch rabbits. After measurement of rectal temperature, the animals were exposed to maximum-pulsed field strengths of 1.5 kV/cm at a PRR of 38 ± 2 Hz for a period of two hours, which resulted in each animal being exposed to a total of $2.73 \times 10^5 \pm 1.44 \times 10^4$ pulses. Immediately following EMP or sham exposure a blood sample was obtained, followed by another sampling 24 hours postexposure. Serum chemistry analyses were performed by the SMA 12/60 autoanalyzer.

Identical procedures were followed in a parallel study of the effects of environmental heat stress on the same serum components. In this case, the experimental animals were either sham-exposed at room temperature or exposed for two hours to an ambient temperature of 40 °C in a temperature-controlled environmental chamber at the Medical College of Virginia. Again, pre-exposed and postexposure rectal temperature was measured.

Using the procedures above, the effects of a two-hour exposure on serum triglyceride levels were evaluated in six EMP-exposed and five sham-exposed Dutch rabbits. Serum triglyceride concentration was determined by the method of Dole [Dole, 1956].

In an attempt to detect tissue-specific effects of EMP exposure, the exposure method described above was used to investigate alterations in the serum levels of MM, MB, and BB fractions of CPK isoenzymes. Isoenzyme levels were determined by the method described by Nealon and Henderson [1975]. Serum samples were taken immediately following a

two-hour EMP or sham exposure. For the purpose of comparing EMP effects on CPK isoenzymes with another type of physiological stress, a comparable group of Dutch rabbits was heat stressed in a temperature-controlled environmental chamber at 40 °C for two hours.

CPK isoenzyme concentrations are affected by storage time between sampling and analysis as well as by the age and metabolic status of the experimental animal. For these reasons, the effects of EMP exposure and heat stress were compared using independent sham exposures for each treatment.

RESULTS

Pentobarbital-Induced Sleeping Time

The results of two independent experiments on the effects of EMP exposure on sodium pentobarbital-induced sleeping time are summarized in Table 1. Experiment 1 consisted of a comparison of effects of exposure to 10 Hz, 1.4 kV/cm fields or to 1.9 kV/cm fields at a PRR of 24 Hz with a group of sham-exposed controls. EMP exposure in both instances resulted in a nonstatistically significant increase in the mean sleeping time. In the second experiment, the effect of exposure to a 0.9 kV/cm, 10-Hz EMP field was compared to sham exposure, the results again indicating a slight nonstatistically significant increase in mean sleeping time in the exposed group. The decrease in the mean rectal temperature noted in all experiments in this series was attributed to anesthesia. No EMP exposure-related effects on rectal temperature were detected.

TABLE 1. Effect of EMP Exposure on Sodium Pentobarbital-Induced Sleeping Time*

Treatment	N	Sleeping time (min)	Rectal temperature change (°C)	Body weight (kg)
Experiment 1				
1.4 kV/cm, 10 Hz	5	53.8 ± 4.0	−0.0 ± 0.1	1.3 ± 0.1
1.9 kV/cm, 24 Hz	5	55.6 ± 6.2	−0.4 ± 0.1	1.4 ± 0.1
Sham-exposure	6	51.2 ± 6.0	−0.6 ± 0.2	1.4 ± 0.1
Experiment 2				
0.9 kv/cm, 10 Hz	5	63.0 ± 9.6	−0.7 ± 0.1	1.7 ± 0.1
Sham-exposure	5	62.4 ± 6.7	−1.0 ± 0.3	1.8 ± 0.1

Values are mean ± SE.
*Dosage, 22 mg/ml.
EMP = electromagnetic pulse; N = number of animals.

Serum Components

The effects of a two-hour EMP exposure, sham exposure, or exposure at an ambient temperature of 40 °C on selected serum components are summarized in Table 2. No statistically significant alterations in serum chemistry resulted from a two-hour EMP exposure at 1.5 kV/cm and a PRR of 38 Hz. Serum enzyme levels of alkaline phosphatase and SGOT were elevated in the immediate postexposure samples, an effect also noted after 2 hours of heat stress, but the variability of the data, attributable in part to the small sample sizes, is such that no statistical significance is attached to these results. Statistically significant differences in mean serum glucose for the pre-exposure base-line samples and the EMP or sham-exposed samples was attributed to the effect of transporting the animals between laboratories. This effect was noted in other serum chemistry values but the dif-

ferences were not found to be statistically significant. As a result of this trip effect, comparisons were made only between treatment and sham responses obtained simultaneously under the same exposure conditions.

The mean and standard error of the mean rectal temperature changes were 0.28 ± 0.21 °C for the EMP-exposed animals and 0.10 ± 0.26 °C for the sham-irradiated controls. EMP exposure under the conditions of this experiment did not result in statistically significant alterations in serum components as compared to sham-irradiated controls. Exposure of Dutch rabbits to an ambient temperature of 40 °C for two hours led to a mean elevation of rectal temperature of 2.1 ± 0.4 °C, a statistically significant elevation relative to the other experimental groups ($P < 0.05$). There was a statistically significant ($P < 0.05$) reduction in serum calcium in heat-stressed rabbits relative to sham-heat-stressed animals.

Serum triglyceride levels were determined in a group of six Dutch rabbits immediately following a two-hour exposure to EMP radiation at a field strength of 1.5 kV/cm and at a PRR of 38 ± 2 Hz, for a total exposure of $2.73 \times 10^5 \pm 1.44 \times 10^4$ pulses. The mean and standard error of the mean serum triglyceride levels were 38.3 ± 4.1 mg/dl as compared to a mean concentration of 40.4 ± 5.9 mg/dl for a group of five sham-irradiated rabbits. Thus, there was no evidence that EMP exposure under the stated conditions altered serum triglyceride levels.

The effect of EMP exposure and heat stress on serum CPK isoenzyme concentrations are summarized in Table 3. The MM fraction and total CPK levels were elevated in the EMP-exposed group, and there was a decrease in the MB and BB levels relative to the sham-irradiated responses. The results were, however, not statistically significant ($P > 0.05$). Although serum CPK isoenzyme levels are known to be altered as a consequence of physiological stress, there are no data on the effects of physical or chemical agents on these isoenzymes in the Dutch rabbits other than the effect of a two-hour heat stress reported here. Heat stress resulted in a 95% increase in MM isoenzyme, 44% increase in MB, 59% increase in BB, and a 75% increase in total serum CPK, indicating a consistent but not statistically significant elevation.

The serum of animals exposed for the CPK study was analyzed for Na^+ and K^+ by flame photometry to determine the effect of EMP exposure. The mean and standard error of the serum sodium concentration for the six Dutch rabbits exposed to the 2 kV/cm, 50-Hz EMP field for two hours were 142.4 ± 3.4 mEq/liter as compared to 138.8 ± 4.9 mEq/liter for the sham-irradiated controls. The difference in the mean Na^+ serum concentrations was not statistically significant, nor was the difference in the mean K^+ concentrations which were 4.3 ± 0.1 and 4.4 ± 0.2 mEq/liter for the EMP-exposed and sham-irradiated controls, respectively.

DISCUSSION

The results of these studies on EMP exposure in the Dutch rabbit do not indicate any consistent, statistically significant effects on the dependent variables investigated. These results are in general agreement with those of other EMP studies on animals [Skidmore and Baum, 1974; Baum et al, 1976; Baum, 1979] and on tissue preparations [Sandler et al, 1975]. Mice, rats, and dogs were exposed for periods of up to 94 weeks to 4.47 kV/cm, 5-Hz EMP pulses with a rise time of 5 ns and a decay (1/e) time of 0.55 μs with no apparent deleterious effects other than transient alterations in some hematological responses [Skidmore and Baum, 1974; Baum et al, 1976; Baum, 1979]. Exposure of bull-

TABLE 2. Effect of EMP Exposure and Heat Stress on Serum Chemistry of Dutch Rabbits

Treatment	N	Ca^{++} (mg %)	Inorganic phosphate (mg %)	Glucose (mg %)	Alkaline phosphatase (mU/ml)	SGOT (mU/ml)
Pre-exposure baseline	10	13.7 ± 0.2	5.0 ± 0.2	191.0 ± 8.2	88.5 ± 13.6	31.7 ± 6.8
Immediate post-2 hr sham exposure	2	14.2 ± 1.3	5.1 ± 0.1	159.5 ± 0.5	61.5 ± 3.5	35.5 ± 17.5
Immediate post-2 hr EMP exposure, 1.5 kV/cm; 38 Hz PRR	3	14.1 ± 0.5	4.5 ± 0.6	153.7 ± 2.9	70.7 ± 11.1	54.3 ± 16.8
1 Day post-sham exposure	2	13.8 ± 0.1	5.1 ± 0.3	150.5 ± 0.5	130.5 ± 7.5	24.5 ± 0.5
1 Day post-EMP exposure	3	14.1 ± 0.6	4.0 ± 0.7	145.0 ± 8.7	75.0 ± 27.1	22.3 ± 2.3
Immediate post-2 hr sham heat stress (22 °C)	4	14.4 ± 0.2*	4.3 ± 0.1	157.3 ± 5.1	39.3 ± 7.8	18.8 ± 2.3
Immediate post-2 hr heat stress (40 °C)	4	13.7 ± 0.1*	3.8 ± 0.3	152.8 ± 6.8	49.0 ± 6.1	27.3 ± 6.1

Values are mean ± SE.
EMP = electromagnetic pulse; N = number of animals; PRR = pulse repitition rate.
*Statistically significant difference in response means as determined by Student's t-test ($P < 0.05$).

TABLE 3. Effect of EMP Exposure and Heat Stress on Rabbit Serum CPK Isoenzymes

Treatment	N	Rectal temperature change (°C)	CPK isoenzyme levels (units/liter)			
			MM	MB	BB	Total CPK
2 hr sham-EMP exposure	6	0.1 ± 0.1	95.3 ± 7.4	12.9 ± 2.4	30.9 ± 10.9	136.0 ± 19.2
2 hr EMP exposure, 2 kV/cm; 50 Hz PRR	6	0.3 ± 0.4	117.6 ± 28.1	12.6 ± 1.4	17.7 ± 2.8	147.3 ± 30.8
2 hr sham heat stress (22 °C)	4	0.1 ± 1.5	55.7 ± 6.5	13.0 ± 1.5	50.7 ± 22.6	119.4 ± 18.0
2 hr heat stress (40 °C)	4	2.1 ± 0.4	108.9 ± 34.7	18.8 ± 5.5	80.3 ± 49.1	208.1 ± 60.2

Values are mean ± SE.
EMP = electromagnetic pulse; PRR = pulse repitition rate; N = number of animals.

frog spinal cord and medulla tissue sections to six 1.3 kV/cm pulses with a width at half-amplitude of approximately 30 ns did not result in gross histological alterations [Sandler et al, 1975].

In contrast to these results, in vitro studies of the effects of pulsed conductive electrical fields on erythrocyte membranes found field-dependent alterations in cell membrane permeability [Sale and Hamilton, 1968; Reimann et al, 1975; Tsong et al, 1976]. The threshold field strength for the induction of permeability changes leading to K^+ efflux was found to be approximately 2 kV/cm and the minimum pulse decay time for this effect, for an exponentially decaying pulse, was on the order of 0.4 μs, regardless of the number of voltage pulses applied to the membrane [Cleary et al, 1977]. Theoretical as well as experimental determinations indicate that such alterations of permeability are induced under exposure conditions that result in insignificant sample heating (ie, < 0.01 °C). Thus, these in vitro results suggest that specific exposure conditions may be required to elicit detectable physiological changes in experimental animals exposed to capacitive EMP fields. Thus, in order to compare in vitro cell membrane permeability field strength or pulse duration thresholds for conductive fields with in vivo responses to capacitive or radiative EMP fields, the fields induced in experimental animals from capacitive fields must be determined.

The electric field induced in an experimental animal exposed to a time-varying capacitive electromagnetic field of the type used in this study may be determined by:

$$E_i = \omega_o \, dE_c/dt, \tag{1}$$

where E_i is the induced field strength, ω_o is a constant determined by the sample conductivity and E_c is the strength of the applied capacitive electrical field [Cleary et al, 1977]. If the EMP, as employed in our experiments is an exponentially decaying pulse with a rise time T_r, the maximum electric-field strength induced at the surface of the experimental animal may be approximated by the relationship,

$$E_i^{max} = E_c^{max} (\omega_o T_r)^{-1}, \tag{2}$$

where $\omega_o = 2.96 \times 10^{10}$ radians $(s)^{-2}$. Thus, for an EMP rise time on the order of 0.1 μs and a maximum applied field strength of 2 kV/cm, the maximum field strength induced at the surface of an experimental animal would be on the order of 1 V/cm. Even allowing for errors in the estimation of the EMP rise time and the sample conductivity, the amplitude of the in vivo-induced electric-field strength is at least one order of magnitude less than the threshold value determined for in vitro cell membrane K^+ permeability alterations, apparently the most sensitive indicant of field-induced functional alterations. The EMP pulse durations employed in this study and in other in vivo studies of a capacitive field [Skidmore and Baum, 1974; Baum et al, 1976; Baum, 1979] were on the order of, or less than, the minimum pulse duration threshold for conductive field-induced alterations in the permeability of erythrocyte membrane. The results of the in vivo experiments are thus not inconsistent with the in vitro results if it is assumed that the in vivo biological endpoints investigated were dependent upon field-induced physical alterations in membrane permeability. Since in vitro studies have been primarily restricted to erythrocyte membrane alterations, the possibility that other cell types such as neurons may exhibit greater sensitivity to pulsed fields cannot be ruled out.

Values are mean ± SE.
EMP = electromagnetic pulse; PRR = pulse repetition rate; N = number of animals.

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